BIOGRAPHICAL SKETCH

11/11/03

NAME C.-K. James Shen

DATE OF BIRTH July 29, 1949

FAMILY Wife Chien Celia Shen
Son Jeffrey Shen

EDUCATION

National Taiwan University	B.S.	1971	Chemistry
University of Calif., Berkeley	Ph.D.	1977	Biological Chemistry
California Institute of Technology	Postdoc	1978-81	Molecular Biology

PROFESSIONAL EXPERIENCE

1981-1983	Assistant Professor, Department of Genetics University of California, Davis
1981-1986	Director and Consultant, Division of Molecular Biology, Advanced Genetics Research Institute (AGRI), Oakland
1986-1987	Visiting Scientist, Institute of Molecular Biology Academia Sinica, Taipei, ROC
1983-1988	Associate Professor, Department of Genetics University of California, Davis
1991-1992	Visiting Professor, Department of Molecular and Cellular Physiology, Stanford University
1988- 1998	Professor of Genetics, Section of Molecular and Cellular Biology, University of California, Davis
1999-	Emeritus Professor, University of California, Davis
1995-	Adjunct Professor, Institute of Genetics, National Yang-Ming University, Taipei, ROC
1995-	Adjunct Professor, Institute of Molecular Medicine, National Taiwan University, Taipei, ROC
2001-	Scientific Advisory Board, Vita Gen Inc.
1995-	Director and Distinguished Research Fellow, Institute of Molecular Biology, Academia Sinica, Taipei, ROC

ACADEMIC HONORS AND AWARDS

Anthony Earle C. Fellowship, 1975-1977
NIH Postdoctoral Fellowship, 1978-1981
NIH Research Career Development Award, 1984-1989
NIH Fogarty Senior International Fellowship, 1986-1987
Distinguished Research Fellow, Academia Sinica, Taipei, ROC, 1995-
NSC Frontier of Science Research Award, Taiwan, ROC, 2000-2005
Academician, Academia Sinica, Taipei, ROC, 2000-
Fellow, AAAS (American Association for the Advancement of Science), 2003-

ADMINISTRATIVE EXPERIENCE

Graduate Student Committees, total 80
Faculty Search Committees
Department and Section Committees (Teaching, Space, Library, Curriculum, Safety, Admission, Fellowship, etc.)
Chair, Seminar Committee (1988-1989)
Academic Senate Committee (1983-1987)
Academic Personnel Committees
Executive Committee, NIH Cellular and Molecular Biology Training Grant (1987-1992)
Chancellor's Liason Committee for the Reorganization of Biological Sciences (1988-1989)

TRAINING RECORD (1981-2003)

15 Ph.D. students
23 Postdoctoral fellows

TEACHING

(Average 50 lectures a year, 1981-1994)

General Genetics
Human Genetics
Molecular Genetics
Eukaryotic Molecular Genetics
Various seminar courses

RESEARCH INTEREST AND EXPERTISE

Eukaryotic Gene Regulation
Genomes and Chromosomes
Mammalian Cell Differentiation and Development
Molecular Evolution

PROFESSIONAL SERVICES

US

NIH Molecular Biology Study Section, US (1993)
NIH Mammalian Genetics Study Section, US (1984-1985)

Review Panel of Biology and Biotechnology, Lawrence Livermore National Laboratory, Livermore, California, US (1993)
Grant Reviewing for Frontier of Sciences Program (Japan/France), US National Science Foundation (NSF), US Department of Agriculture (USDA), and US Sea Grant Foundation

Taiwan
Executive Scientist, National Genomic Medicine program, Taiwan, ROC (2001-)
Advisory Committee, National Program on Genome and Medicine, National Science Council, Taiwan, ROC (1998- 2001)
Advisory Committee, Molecular and Genome Medicine Core Laboratory, National Health Research Institute, Taiwan, ROC (1996-)(chair, 1996-1999)
Advisory Committee, Institute of Molecular Biology, Academia Sinica, Taiwan, ROC (1997-)
Advisory Committee, Biotechnology Program in Medicine, Academia Sinica, Taiwan, ROC (1995-)
Advisory Committee, Institute of BioAgricultural Sciences, Taiwan, ROC (1998-2001)
Advisory Committee, Institute of Zoology, Academia Sinica, Taiwan ROC (1993-1996)
Panel, Biology Program Grants, National Science Council, Taiwan, ROC (1996-)
Honorary Advisor, Veteran Hospital, Taipei, ROC (1995-)
Advisory Committee, Development Fund, Executive Yuan, Taiwan, ROC (1998-2002)
The Board of Directors, Tunghai University, Taiwan, ROC (2003-)
Advisory Committee, Tunghai University Life Science Research Center, Taiwan, ROC (2003-)
Advisory Committee, Genomic Research Center, Academia Sinica, Taiwan, ROC (2003-)
Advisory Committee and Steering Committee, Brain Research Center, National Yang Ming University, Taiwan, ROC (2003-)
Advisory committee, Academic Affairs Committee, National Taiwan Normal University, Taiwan, ROC (2003-)
Advisory Committee, Academic Affairs Committee, Ministry of Education, Taiwan, ROC (2003-)
Advisory Committee, Science Education Committee, Ministry of Education, Taiwan, ROC (2003-)

EDITORIAL SERVICE

Editorial Board, Analytical Biochemistry (1981-1987), Molecular and Cells (2003-)
Ad Hoc Manuscript Reviewers for Journals: Analytical Biochemistry, Biochem. Biophys. Res. Comm., Biochem. Biophys. Acta,, British J. of Hematology, Development , Gene, Genomics, J. Biol. Chem., J. Mol. Evol., Mol. Cell Biol., Nature, Nucleic Acids Res., Proc. Natl. Acad. Sci. USA, Science, Trents in Genetics

PROFESSIONAL ASSOCIATIONS

Academician, Academia Sinica, Taipei, ROC (2000-)
American Society of Biochemistry and Molecular Biology
International Molecular Biology Network (IMBN) (2002~)
Council Member, Society of Chinese Bioscientists in America (1999-2003)
President, Society of Molecular and Cell Biology, Taiwan, ROC (1997-2001)

President, Society of Genetics, Taiwan, ROC (1999-2000)
President, Asian Pacific Society of Biochemistry and Molecular Biology (1999-2001)
Society of Genetics, Taiwan, ROC
Society of Biochemistry, Taiwan, ROC
AAAS
Sigma χi, US

INVITED SEMINARS, CONFERENCE AND SYMPOSIUM TALKS

1977-2003/6/30

International	72
Taiwan	31

Samples

International Symposium on Nucleic Acids, Frederick Cancer Center, Bethesda, May, 1981

Fifty-first Cold Spring Harbor Symposium on Quantitative Biology Molecular Biology of Homo Sapiens. May 28-June 4, 1986

Biological Sciences Series, Department of Biochemistry, Johns Hopkins University. May, 1986

Department of Biochemistry and Biotechnology Institute, University of British, Columbia, Vancouver, Canada, Sept. 1988

Department of Mammalian Cell Expression, AMgene, Thousand Oaks, California, Nov. 7, 1989

Molecular and Cell Biology Training Program, University of Maryland, Dec. 1990

Session Chair and Speaker, Sixth International SCBA Conference, Vancouver, Canada, June 23-30, 1995

Session Chair, 10th Conference of Hemoglobin Switch, Seattle, Washington, June 1996
Department of Pharmacology, University of Southern California, Los Angeles, California, July 21, 1997

Department of Ecology and Evolution, University of Chicago, Chicago, Nov. 18, 1997

Beckman Research Institute, City of Hope Hospital, Los Angeles, California, Dec. 5, 1997

Dept. of Biology, Yale University. Dec. 1998

Center of Excellence International Symposium on Molecular Evolution of Primates, Kyoto University, Japan. Nov. 17-21, 1999

Dept. of Molecular, Cell & Development Biology, UCLA, California, USA, April 26, 2001

Gordon Conference on Red Cells, New Hampshire, July 22-27, 2001

The 13th International Conference on Hemoglobin Switching, St. John's College, Oxford, September 26-30, 2002

The Korea-Japan Drosophilists' Symposium, Seoul, Korea, Oct. 17-18, 2002

Keynote, The International Symposium of Comparative Genomics, National Yang-Ming University, Taiwan, ROC, Nov. 17th. 2002

Gordon Conference on Red Cells, IL Ciocco, Italy, May 25-30, 2003

The First Symposium on Frontiers of Biomedical Science, Epigenetics in Development and Disease, Shanghai, China, October 24-26,2003

Xiangshan Science Symposium on Genomics & Evolution, Beijing, China, October 27-31,2003

GRANT SUPPORT

Past

American Cancer Society, "Molecular Genetic of Human Actin Gene Family" (1984-1986)

California Biotechnology Training Grant, "DNA Supercoiling in Mammalian cells" (1986)

NIH Research Career Development Award, US Public Health (1984-1989)

NIH, US Public Health, "Evolution and Regulation of Primate Globin Gene Families" (1981- 1999)

National Science Council Grant, Taiwan, ROC "Erythroid Gene Regulation" (1995-1999)

Current

Frontier of Science Research Award, NSC, Taiwan, ROC "DNA CpG MTases and DNA methylation in Eukaryotic Development" (2000-2005)

National Health Research Institute, Taiwan, ROC "Human Globin Gene Switch" (1996-2006)

National Science Council/ Academia Sinica Genomics Program, "Novel Genes in Mammalian Brain Structure and Function" (2002-2005)

PUBLICATIONS

1. Shen, C.-K.J., Wiesehahn, G. and Hearst, J.E.. (1976) Cleavage Patterns of Drosophila melanogaster satellite DNAs by restriction enzymes. Nucleic Acids Res. 3, 931-952.

2. Hanson, C.V., Shen, C.-K.J. and Hearst, J.E. (1976) In situ crosslinking of DNA as a probe for chromatin structure. Science 193, 62-64.

3. Shen, C.-K.J. and Hearst, J.E. (1976) Psoralen cross-linked secondary structure map of single-stranded virus DNA. Proc. Natl. Acad. Sci. USA 73, 2649-2653.

4. Shen, C.-K.J. and Hearst, J.E. (1977) Detection of long range sequence order in Drosophila melanogaster satellite DNA IV by photochemical reaction and denaturation microscopy. J. Mol. Biol. 112, 495-507.

5. Isaccs, S.T., Shen, C.-K.J. Hearst, J.E. and Rapoport, H. (1977) Synthesis and characterization of new psoralen derivatives with superior photoractivity with DNA and RNA. Biochem. 16, 1058-1064.

6. Shen, C.-K.J. and J.E. Hearst. 1977. Mapping of sequences with two-fold symmetry on the simian virus 40 genome: a photochemical cross-linking approach. Proc. Natl. Acad. Sci. USA 74:1363-1367.

7. Shen, C.-K.J. and Hearst, J.E. (1977) Chromatin structures of mainband and satellite DNAs in Drosophila melanogaster nuclei as probed by photochemical cross-linking of DNA with troxsalen. Cold Spring Harbor Symposium Quantitative Biology XLII, 179-189.

8. Shen, C.-K.J., Hsieh, T.-S., Wang, J.C. and Hearst, J.E. (1977) Photochemical cross-linking of DNA-RNA helices by psoralen derivatives. J. Mol. Biol. 116, 661-679.

9. Shen, C.-K.J. and Hearst, J.E. (1978) Photochemical cross-linking of transcription complexes with psoralen. I. Covalent attachment of in vitro SV40 nascent RNA to its double-stranded DNA template. Nucleic Acids. Res. 5, 1429-1441.

10. Shen, C.-K.J., Ikoku, A.S. and Hearst, J.E. (1979) A specific DNA orientation in the filamentous bacteriophage fd as probed by psoralen cross-linking and electron microscopy. J. Mol. Biol. 127:163-175.

11. Shen, C.-K.J. and Hearst, J.E. (1979) A technique for relating long range base pairing on single-stranded DNA and eukaryotic RNA processing. Anal. Biochem. 95,108-116.

12. Lauer, J., Shen, C.-K.J. and Maniatis, T. (1980) Chromosomal arrangement of human α-like globin genes: sequence homology and α-globin gene deletion. Cell 20, 119-130.

13. Shen, C.-K.J. and Maniatis, T. (1980) Tissue specific DNA methylation in cluster of rabbit β-like globin genes. Proc. Natl. Acad. Sci. USA. 77, 6634-6638.

14. Shen, C.-K.J. and Maniatis, T (1980) The organization of repetitive sequences in a cluster of rabbit β-like globin genes. Cell 19, 379-391.

15. Fritsch, E.F., Shen, C.-K.J. Lawn, R.M. and Maniatis, T. (1981) The organization of repetitive sequence in mammalian globin gene clusters. Cold Spring Harbor Symposium Quantitative Biology 45, 762-775.

16. Shen, C.-K.J. and Maniatis, T. (1982) The organization, structure and in vitro transcription of Alu family RNA polymerase III transcription units in the human α-like globin gene cluster: Precipitation of in vitro transcripts by lupus anti-la antibodies. J. Mole. Appl. Genet. 1, 343-360.

17. Shen, C.-K.J. and Maniatis, T. (1982) Nucleotide sequence, DNA modification and in vitro transcription of Alu family repeats in the human α-like globin gene cluster. In Genetic Engineering Techniques Recent Developments, eds. R.C. Huang, T.T. Kuo, and R. Wu, Academic Press, 129-158.

18. Fox. F.M., Hess, J.F. Shen, C.-K.J. and Schmid, C.W. (1983) Alu family members in the human α-like globin gene cluster. Cold Spring Harbor Symposium of Quantitative Biology 47, 1131-1134.

19. Hess, J.F., Fox, G.M., Schmid, C.W. and Shen, C.-K.J. (1983) Molecular evolution of the human adult α-like globin gene region - insertion and deletion of the Alu family repeats and non-Alu DNA sequences. Proc. Natl. Acad. Sci. USA 80, 5970-5974.

20. Shen, C.-K.J. (1983) Superhelicity induces hypersensitivity of a human polypyrimidine polypurine DNA sequence in the human α2-α1 globin intergenic region to S1 nuclease digestion-high resolution mapping of the clustered cleavage sites. Nucleic Acids Res. 11, 7899-7910.

21. Sawada, I., Beal, M.P., Shen, C.-K.J., Chapman, B., Wilson, A.C. and Schmid, C. (1983) Intergenic DNA sequences flanking the pseudo α globin genes of human and chimpanzee. Nucleic Acids Res. 11, 8087-8101.

22. Perez-Stable, C., Ayres, T.M. and Shen, C.-K.J. (1984) Distinctive sequence organization and functional programming of an Alu repeat promoter. Proc. Natl. Acad. Sci. USA 81, 5291-5295.

23. Hess, J.F., Schmid, C. and Shen, C.-K.J. (1984) Gradient of sequence divergence in the human adult α-globin duplication units. Science 226, 7-10.

24. Shen, C.-K.J. (1985) DNA methylation and developmental regulation of eukaryotic globin gene transcription. In DNA methylation, eds. A. Riggs, H. Cedar and A. Razin. Springer-Verlag, pp. 249-268.

25. Schmid, C. and Shen, C.-K.J. (1985) The evolution of interspersed repetitive DNA sequences in mammals and other vertebrates. In: Molecular Evolution Genetics, ed. R.J. McIntryre. Plenum Publ. Inc. pp. 323-358.

26. Hess, J.F., Perez-Stable, C., Wu, G., Weir, B., Tinoco, I. Jr. and Shen, C.-K.J. (1985) A new type of RNA polymerase III-dependent transcriptional terminator: biochemical and evolutionary implications. J. Mol. Biol. 184,7-21.

27. Hess, J.F., Perez-Stable, C., Deisseroth, A. and Shen, C.-K.J. (1985) Characterization of an unique RNA initiated upstream from the human α1-globin gene--polymerase II dependence, tissue specificity, and subcellular distribution. Nucleic Acids Res. 13, 6059-6074.

28. Willard, C., Wong, E., J., Hess, F. C.-K.J. Shen, Chapman, B., Wilson, A.C. and Schmid, C.W. (1985) Comparison of human and chimpanzee ξ1 globin genes. J. Mol. Evol. 22, 309-315.

29. Sawada, I., Willard, C., Shen, C.-K.J., Chapman, B., Wilson, A., and Schmid, C.W. (1985) Evolution of alu family repeats since the divergence of human and chimpanzee. J. Mol. Biol. 22, 316-322.

30. Shen, C.-K.J. and Hu, W.-S. (1986) DNA supercoiling of recombinant plasmids in mammalian cells. Proc. Natl. Acad. Sci. USA 83, 1641-1645.

31. Marks, J., Shaw, J.-P. and Shen, C.-K.J. (1986) The orangutan adult □-globin gene locus: Duplicated functional genes and a new member of the primate □-globin gene family. Proc. Natl. Acad. Sci. USA 83, 1413-1417.

32. Perez-Stable, C. and Shen, C.-K.J. (1986) Competitive and cooperative functioning of the two promoter elements of a human Alu family repeat. Mol. Cell. Biol. 6, 2041-2052.

33. Marks, J., Shaw, J.-P. and Shen, C.-K.J. (1986) The primate alpha globin-like gene θ1: Novel sequence organization and genomic complexity. Nature 321, 785-788.

34. Marks, J., Shaw, J.-P. Perez-Stable, C., Hu, W.-S., Ayres, T.M., Shen, C.C. and Shen, C.-K.J. (1986) The primate α globin family: A paradigm of genomic fluidity. Cold Spring Harbor Symposium Quantitative Biology 51, 499-508.

35. Hu, W.-S. and Shen, C.-K.J. (1987) Reconstruction of human α-thalassemia genotypes in monkey cells. Nucleic Acids. Res. 15, 2989-3008.

36. Shaw, J.-P., Marks, J. and Shen, C.-K.J. (1987) Evidence that the recently discovered θ1 gene is functional in higher primates. Nature 326, 717-720.

37. Shaw, J.-P., Marks., J., Mohandas, T., Sparkes, R. and Shen, C.K.J. (1987) The adult αglobin gene loci from monkey to man. In Prog. Clin. Biol. Res., eds. A. Nienhuis and G. Stamatoyannopoulos, Alan R. Liss, Inc., NY, 251, 65-80.

38. Gomez-Pedrozo, M., Mohandas, T., Sparkes, R., Shaw, J.-P., Hess, J.F., Ayres, T.M. and Shen, C.-K.J. (1987) Evolution of human cytoplasmic actin gene sequences: chromosomal mapping and structural characterization of three cytoplasmic actin-like pseudogenes including one on the Y chromosome. J. Human Evolution 16, 215-230.

39. Hsu, S.-L., Marks, J., Shaw, J.-P., Tam, M., Higgs, D.R., Shen, C.C. and Shen, C.-K.J. (1988) Structure and expression of human θ1 globin gene. Nature 331, 94-96.

40. Perez-Stable, C., Shen, C.C. and Shen, C.-K.J. (1988) Enrichment and depletion of Hela toposimerase I recognition sites among specific types of DNA elements. Nucleic Acids. Res. 16, 7973-7993.

41. Gomez-Pedrozo, M., Hu, W.-S. and Shen, C.-K.J. (1988) Recombinational resolution in primate cells of two homologous human DNA segments with a gradient of sequence divergence. Nucleic Acids Res. 16, 11237-11247.

42. Shaw, J.-P., Marks, J., Shen, C.C. and Shen, C.-K.J. (1989) Anomalous and selective DNA mutations of the Old World Monkey α globin genes. Proc. Nat. Acad. Sci. USA 86, 1312-1316.

43. Shen, C.C., Bailey, A., Kim, J.-H., Yuan, C.-Y., Marks, J., Shaw, J.-P., Klisak, I., Sparkes, R. and Shen, C.-K.J. (1989) The human α2-α1-θ1 globin locus: some thoughts and recent studies of its evolution and regulation. In Prog. Clin. Biol. Res., eds. A. Nienhuis and G. Stamatoyannopoulos, Alan R. Liss, Inc., NY 316B, 19-32.

44. Kim, J.-H., Yu, C.-Y., Bailey, A., Hardison, R. and Shen, C.-K.J. (1989) Unique sequence organization and erythroid cell-specific factor-binding of mammalian θ1 globin promoter. Nucleic Acids Res. 17, 5687-5700.

45. Shen, C.-K.J. (1989) Molecular evolution of higher primates: The α2-α1-θ1 locus. In The Molecular Evolution, ICN-UCLA Symposium, eds. M.T. Clegg and S.J. O'Brien, Alan R. Liss, Inc., pp. 75-83.

46. Yu, C.-Y., Chen, J., Lin, L.-I., Tam, M. and Shen, C.-K.J. (1990) Cell-type specific protein-DNA interactions in the human ξ globin upstream promoter region: Displacement of Sp1 by the erythroid specific factor of NF-E1. Mol. Cell. Biol. 10, 282-294.

47. Shen, C. and Shen, C.-K.J. (1990) Specificity and flexibility of the recognition of DNhelical structure by eukaryotic topoisomerase I. J. Mol. Biol. 212, 67-78.

48. Yu, C.-Y., Motamed, K., Chen, J., Bailey, A.D. and Shen, C.-K.J. (1991) The CACC box upstream of human embryonic ε globin gene binds Sp1 and is a functional promoter element in vitro and in vivo. J. Biol. Chem. 266, 8907-8915.

49. Shaw, J.-P., Marks, J. and Shen, C.-K.J. (1991) The adult α-globin locus of Old World monkeys: An abrupt breakdown of sequence similarity to human is defined by an insertion site of human Alu family repeat. J. Mol. Evol. 33, 506-513.

50. Reddy, S. and Shen, C.-K.J. (1991) Protein-DNA interactions in vivo of an erythroid-specific, human β-globin locus enhancer. Proc. Natl. Acad. Sci. USA. 88, 8676-8680.

51. Bailey, A.D., Stanhope, M., Slightom, J.L., Goodman, M., Shen, C.C. and Shen, C.-K.J. (1992) Tandemly duplicated α globin genes of gibbon. J. Biol. Chem. 267, 18398-18406.

52. Chu, C. and Shen, C.-K.J. (1993) DNA methylation: Its possible functional roles in developmental regulation of human globin gene families. In DNA Methylation: Its Biological Significance, eds. Host, J.-P. and Salaz, H.P., Berkhauser Verlag, pp. 385-403.

53. Motamed, K., Bastiani, C., Zhang, Q., Bailey, A.P. and Shen, C.-K.J. (1993) CACC box and enhancer response of the human embryonic ε globin promoter. Gene 123, 235-240.

54. Reddy, S. and Shen, C.-K.J. (1993) Erythroid differentiation of MEL cells results in re-organization of protein-DNA complexes in the mouse β^{maj} globin promoter but not its distal enhancer. Mol. Cell Biol. 13, 1093-1103.

55. Zhang, Q., Reddy, S., Yu, C.-Y., Bastiani, C., Higgs, D., Stamatoyannopoulos, G., Papayannopoulou, T. and Shen, C.-K.J. (1993) Transcriptional activation of human ζ2 globin promoter by the α globin regulatory element (HS-40): Functional role of specific nuclear factor-DNA complexes. Mol. Cell Biol. 13, 2298-2308.

56. Bailey, A.D. and Shen, C.-K.J. (1993) Sequential insertion of the Alu family repeats into specific genomic sites of higher primates. Proc. Natl. Acad. Sci. USA 90, 7205-7209.

57. Reddy, S., Stamatoyannopoulos, G., Papayannopoulou, T., and Shen, C.-K.J. (1994) Genomic footprinting and sequencing of human β-like globin gene locus: Tissue specificity and cell like artifact. J. Biol. Chem. 269, 8287-8295.

58. Zhang, Q., Rombel, I., Reddy, G. N., Gang, J.-B. and Shen, C.-K.J. (1995) Functional roles of in vivo footprinted DNA motifs within an α-globin enhancer. J. Biol. Chem. 270, 8501-8505.

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60. Zhang, Q., Rombel, I., Reddy, G.N. and Shen, C.-K.J. (1995) Transcriptional regulation of human ζ2 and α globin promoters by multiple nuclear factor-DNA complexes: The final act. In: *Molecular Biology of Hemoglobin Switch,* ed. Stamatoyannopoulos, G., Intercept Limited, pp. 193-202.

61. Jimenez-Ruiz, A., Zhang, Q. and Shen, C.-K.J. (1995) In *vivo* binding of trimethylpsoralen detects structural alterations associated with transcribing regions in the human β-globin cluster. J. Biol. Chem. 270, 28978-28981.

62. Lin, L.-I., Lin, K.-S. and Shen, C.-K.J. (1996) Current Status of Thalassemia in Taiwan. J. Genet. Mol. Biol. Taiwan. 7, 25-30.

63. Bailey, A.D., Shen, C.C. and Shen, C.-K.J. (1997) Molecular origin for the mosaic sequence arrangements of higher primate alpha globin duplication units. Proc. Natl. Acad. Sci. USA. 94, 5177-5182.

64. Gavva, N. R., Gavva, R., Ermekova, K., Sudol, M. and Shen, C.-K.J. (1997) Interaction of WW domains with hematopoietic transcription factor p45/NF-E2 and RNA polymerase II. J. Biol. Chem. 272, 24105-24108.

65. Huang, B.-L., Fan-Chiang, I.R., Wen, S. C., Koo, H.-C., Kao, W. Y., Gavva, N.R. and Shen, C.-K. James (1998) Derepression of human embryonic ζ-globin promoter by a locus-control region sequence. Proc. Natl. Acad. Sci. USA. 95, 14669-14674.

66. Daftari, P., Gavva, N.R. and Shen, C.-K. J. (1999) Distinction between AP1 and NF-E2 factor-binding at specific chromatin regions in mammalian cells. Oncogene. 18, 5482-5486.

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68. Hung, M.-S., Karthikeyan, N., Huang, B.-L., Koo, H.-C., Kiger, J. and Shen, C.-K. J. (1999) Drosophila proteins related to vertebrate CpG MTases. Proc. Natl. Acad. Sci. USA. 96, 11940-11945.

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73. Shen, C.-K.J. (2001) Sharing duties in the family. Genome Research, 11, 1615.

74. Moreau-Gaudry, F., Xia, P., Jiang, G., Bauer, G., Ellis, J., Surinya, K. H., Mavilio, F., Shen, C.–K.J. and Malik, P. (2001). High level erythroid-specific gene expression in primary human and murine hematopoietic cells with self-inactivating lentiviral vectors. Blood , 98, 2664-2672..

75. Gavva, N. R., Wen, S.C., Daftari, P., Moniwa M., Yang, W.M., Yang-Feng, L.-P. Teresa, Seto, E., Davie, J. R. and Shen, C.-K. J. (2002). NAPP2/Pex14p, a peroxisomal membrane protein is also a transcriptional corepressor. Genomics, 79, 423-431.

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PATENT
Docket No. 514162000120

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Che-Kun James SHEN

Serial No.: 10/014,220

Filing Date: November 9, 2001

For: HS-40 ENHANCER-CONTAINING VECTORS IN TRANSGENIC ANIMALS

Examiner: S. Kaushal

Group Art Unit: 1636

DECLARATION OF CHE-KUN JAMES CHEN PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Che-Kun James Shen, declare as follows:

1. I am currently employed as a Distinguished Research Fellow and Director at the Institute of Molecular Biology, Academia Sinica.

2. I am the inventor of the invention disclosed in the above-referenced patent application, and am familiar with the contents thereof. I have assigned my rights in the invention to the Academia Sinica and stand to receive 20% of profits in connection with the invention pursuant to my employment with Academia Sinica.

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3. I received a Ph.D. in Chemistry from the University of California, Berkeley, July, 1977, and have been actively involved in molecular biology and biotechnology-related research for 30 years. My curriculum vitae is attached hereto as Exhibit A.

4. My laboratory has been generating transgenic animals since 1997. As of the filing of the application for the claimed invention on December 4, 1998, it is my opinion that generation of transgenic animals was a routine procedure in the art. We use protocols that are standard in the field. As of December 4, 1998, the standard protocol for generating transgenic animals was microinjection of large number of oocytes with the DNA to be integrated and then implantation into animals to allow the embryos to develop to term. A review of the current skill in the art by Wall, 2001, supports my view that this was a routine protocol. Wall reports: "[Production of transgenic animals by pronuclear microinjection] is a known technology, which is to say there is a reasonable chance of predicting the outcome of experiments...At present pronuclear microinjection...is the least complicated, reliable method available for producing transgenic animals... Experience has taught us that once microinjection skills are perfected there are only a few parameters one needs to be concerned about to successfully produce transgenic animals."

5. The efficiency of integration using this method was and still is low, but that low efficiency is simply part of the overall protocol and is well accepted and understood in the art. See ,e.g., Wall, 2001, p. 213. By injecting large numbers, the experimenter was virtually assured of obtaining one or more transgenic animals, and reports for transgenic livestock have quoted rates as high as 5 to 10 % of offspring born. Wall, 2001, p. 213. In my lab, we routinely get 10 % in mice and 14 % in pigs. The protocols for a number of animals had been worked out as of the filing of the application. Application of those standard protocols to other animals would only require routine experimentation to modify the protocols if any modification would be required.

6. I have read and understand the following references: Chung, et al, 1997; Namciu, et al, 1998; Pikaart, et al, 1998; Potts, et al, 2000; Willoughby, et al; 2000; van der Vlag, et al 2001; Szabo, et al, 2002 and Frazar, et al, 2003. The references all relate to genetic elements that provide position independent expression when coupled with a promoter. Table A below shows how these references demonstrate that numerous position independence elements are effective in

cross-species experiments. In these references, chicken position-independence elements were shown to be effective in mice, human elements functioned in Drosophila cells, chicken elements functioned in human cells, Drosophila elements functioned in human cells and human elements functioned in mice. For instance a Human Matrix Attachment (MAR) element combined with a Drosophila *white* gene and Drosophila *white* promoter for eye color resulted in position independence insulation in Drosophila by the human MAR element. See Table A. Thus across multiple animal phyla and animal classes, isolated genetic elements still exert their position-independence effects, even when coupled with multiple types of genes and promoters. Additionally the chicken beta-globin HS-4 element from which HS-40 is derived has been particularly shown to function in multiple species including mouse and human, in combination with promoters from widely diverse species of organisms. Its functionality appears to be highly conserved across multiple vertebrate classes. See Szabo, et al, 2002, p. 898.

Table A. References Which Cite Genetic Elements with Cross-Species Position-Independent Effects

Element	**Promoter**	**Gene**	**Element From Species**	**To Species**	**Effect**	**Reference**
5'HS4 One copy of insulator on either side of neo gene	Human gamma globin	Neo	chicken	Human erythroid cell line	insulator effect insulated (from murine beta-globin locus control element)	Chung
Human apoB MAR matrix attachment region (MAR) from apolipo-protein B	*Drosophila white* proximal promoter	*white*	Human	*Drosophila*	Insulator, but not enhancer in *Drosophila*	Namciu, et al.

Chicken beta-globin enhancer	Chicken beta-globin promoter	(human?) IL-2 Receptor tac fragment	Chicken	Chicken erythroid cell line	Two flanking copies resulted in insulator effect	Pikaart
Chicken beta-globin 5'HS4 One copy of insulator on either side of minigene	Murine tyrosinase	Murine tyrosinase minigene	Chicken	mouse	Insulator	Potts
Human Alu2 fragment	Mouse metallo-thionein	Human growth hormone	Human	Mouse	Enhancer blocking effect	Willoughby
Drosophila scs insulator Human MAR	Minimal heat shock inducible promoter	LexA	*Drosophila* and human	Human U-2 OS cell line	*Drosophila* scs insulated repression	Van der Vlag
Chicken beta-globin Insulator Unit Two copies in a row	Mouse H19 promoter	Mouse H19/IGF-2 (insulin like growth factor 2) Mouse	Chicken	Mouse	Insulator	Szabo, et al.
Chicken beta-globin 5'HS4 One copy of insulator on either side of promoter - gene comb.	Mouse Anion Exchanger Protein 1 (AE1) promoter	Mouse Gamma globin	Chicken	Mouse	Insulator/ suppression of position effect variegation	Frazar, et al.

7. Therefore, based upon these references, the transgenic mice and pigs with HS-40 elements generated in my lab, and my experience in the field, it is my opinion that the HS-40 element will function across multiple species. Furthermore, it is my opinion that the function of HS-40 is independent of the particular promoter selected, and therefore HS-40 will provide position independence expression with most if not all promoters. Finally, it is my opinion that the function of HS-40 is independent of the particular transcript expressed and therefore HS-40 will provide position independence expression with most if not all transcripts.

November 21, 2003

Che-Kun James Shen